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Two new marasmielloid fungi widely distributed in the Republic of Korea

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Abstract — Two species of the genus *Marasmiellus*, *M. koreanus* and *M. rhizomorphigenus,* are described as new taxa from the Republic of Korea. Both have been recorded several times during the past years. Their systematic positions are supported through DNA analyses.

Key words — euagarics, DNA studies

Introduction

During joint field excursions sponsored by the Czech-Korean project, "Phylogenetic taxonomy of *Marasmius* (*Basidiomycota*, *Marasmiaceae*) and related genera in the Republic of Korea", several interesting marasmioid, marasmielloid, and gymnopoid fungi have been collected. Some results have already been published (Antonín et al. 2009a,b, 2010). The two new marasmielloid taxa presented here were rather frequently found at several South Korean localities.

Materials and methods

Macroscopic descriptions of collected specimens are based on fresh basidiocarps and made by the first author. Microscopic features are described from dried material mounted in H_2O, KOH, Melzer's reagent, and Congo Red using an Olympus BX-50 light microscope with a magnification of 1000×. For basidiospores, the factors E (quotient of

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length and width in any one spore) and Q (mean of E-values) are used. For lamellae, L stands for the number of entire lamellae and l for the number of lamellulae between each pair of entire lamellae. Authors of fungal names are cited according to the International Plant Names Index Authors website (http://www.ipni.org/ipni/authorsearchpage.do), and colour abbreviations follow Kornerup & Wanscher (1983). Herbarium specimens of the studied fungi are preserved in the herbarium of the Moravian Museum, Brno, Czech Republic (BRNM).

DNA extraction, PCR amplification of ITS and LSU regions of ribosomal DNA, sequencing, and sequence alignment methods followed Antonín et al. (2010). Phylogenetic analyses were made using Bayesian modelling (Geyer 1991) performed with MRBAYES, version 3.0b4 (Ronquist & Huelsenbeck 2003). For a given data set, the general time reversible (GTR) model as selected with Modeltest v 3.06 (Posada & Crandall 1998) was employed with gamma-distributed substitution rates. Markov chains were run for 2,000,000 generations, saving a tree every 100th generation. Among these, the first 1000 trees were discarded as the burn-in phase of each analysis. MRBAYES was used to compute a 50 % majority rule consensus of the remaining trees to obtain estimates for the posterior probabilities (PPs) of the groups. Two species of *Marasmius*, *M. rotula* and *M. capillaris*, were selected as outgroup taxa for rooting purposes.

Taxonomy

Marasmiellus koreanus Antonín, R. Ryoo & H.D. Shin, **sp. nov.** Fig. 1

MycoBank MB 516550

NCBI accession numbers: BRNM 714972 [GU319113 (ITS), GU319117 (LSU)]; BRNM 718782 [GU319114 (ITS), GU319118 (LSU)]

Pileo 27–60 mm lato, hemisphaerico usque ad planum-convexum, centro leviter depresso, subtiliter tomentoso, sulcato, griseo-aurantiaco, brunneo-aurantiaco vel brunneo. Lamellis distantibus, pallide luteis vel aurantiaco-albidis. Stipite 14–70 × 2–3.5(–5) mm, furfuraceo, albido, pallide luteo vel aurantiaco-albido. Basidiosporis 7.5–10(–11) × (3.5–)4.0–5.0(–5.5) µm, fusiformibus, ellipsoideo-fusiformibus vel ellipsoideis, hyalinis, inamyloideis. Cheilocystidiis 25–55 × 4.0–10 µm, cylindraceis, clavatis, fusiformibus vel subutriformibus, irregularibus, coralloideis vel submoniliformibus. Pileipellis ex hyphis cylindraceis, incrustatis, laevibus vel disperse diverticulatis constitua. Caulocystidiis 18–70(–105) × (4.0–)6.0–10 µm, cylindraceis, clavatis, subulatis, fusiformibus, iterum diverticulatis. Hyphis fibulatis, indextrinoideis. Ad ramulos putridos.

Holotypus: *Korea meridionalis, Chiaksan, Wonju, 19. VII. 2009 leg. V. Antonín (09.125) et R. Ryoo (**holotypus** in herbario BRNM 718782 preservatur).*

Basidiocarps single or in groups. Pileus 27–60 mm broad, hemispherical with plane to (slightly) depressed centre, then plano-convex with almost applanate to slightly depressed centre and with low and obtuse central umbo within this depression, margin inflexed and crenulate, undulate when old, finely (fibrillose) tomentose especially at centre, except for smooth centre distinctly radially rugulose-sulcate and finely innately fibrillose (under a lens), translucently striate when moist, greyish orange, brownish orange or brown (6B4–C5, 6–7C5, 7E7) with paler, almost whitish margin. Lamellae distant,

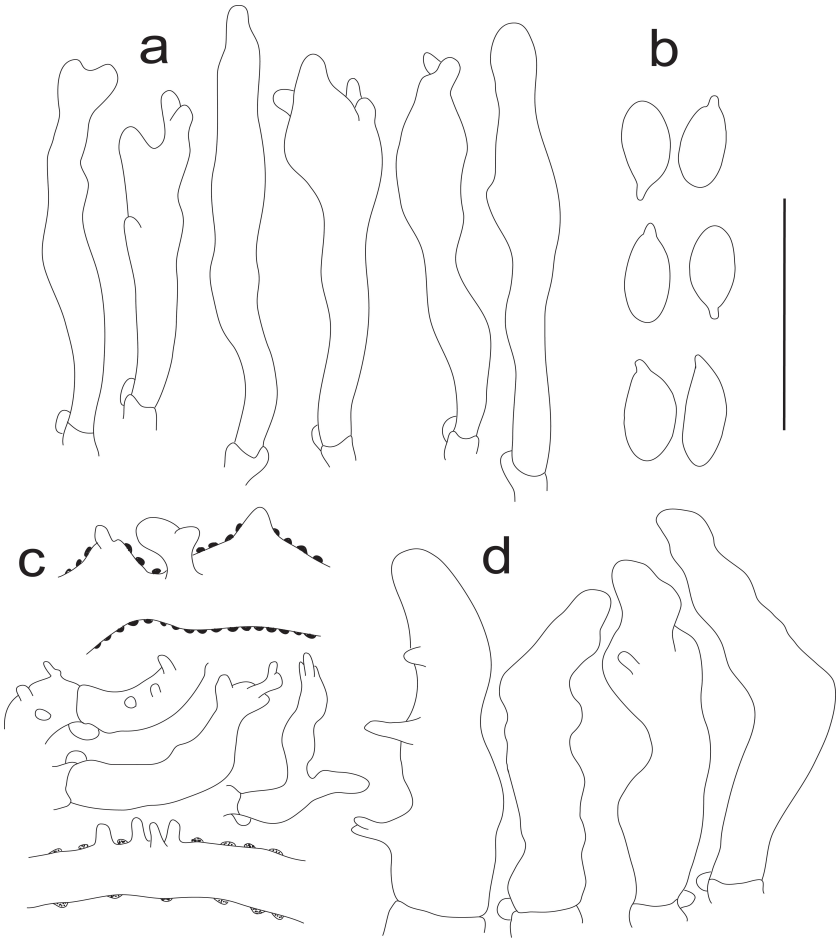

Fig. 1. *Marasmiellus koreanus*.
a. cheilocystidia, b. basidiospores, c. pileipellis hyphae, d. caulocystidia.
Scale bar = 20 μm.

L = 15–20, l = (1–)2–3 (irregular), ± broadly adnate with tooth, ± arcuate when young, slightly intervenose towards pileus margin, light yellow to orange white (4–5A2, 4A3), with concolorous, finely pubescent edge. Stipe 14–70 × 2–3.5 (–5.0) mm, cylindrical or slightly tapering towards base, sometimes laterally compressed (especially when old), slightly broadened above, subbulbous at base, (sub)insititious, longitudinally fibrillose, entirely furfuraceous especially when young, later ± tomentose-furfuraceous especially in upper part, whitish to light yellow to orange-white (± lamellae colour); with whitish basal hairy

tomentum descending to the substrate (± 1 mm). Context membranaceous, whitish, hollow in stipe, without special smell, taste mild.

Basidiospores 7.5–10(–11) × (3.5–)4.0–5.0(–5.5) µm, average = 8.7 × 4.4 µm, E = 1.6–2.4(–2.5), Q = 1.8–2.0(–2.2), fusoid, ellipsoid-fusoid or (broadly) ellipsoid, smooth, hyaline, thin-walled, non-dextrinoid. Basidia 27–40 × 8.0–10 µm, 4-, rarely 2-spored, clavate. Basidioles 15–35(–45) × 3.0–10 µm, cylindrical, clavate or fusoid. Cheilocystidia 25–55 × 4.0–10 µm, variable in shape, cylindrical, clavate, fusoid, subutriform, irregular, lobed, sometimes rostrate, with broad, obtuse projection(s), coralloid or submoniliform, thin- to slightly-walled. Trama hyphae ± cylindrical, thin- to slightly thick-walled, non-dextrinoid, up to 10(–15) µm wide. Pileipellis a cutis composed of cylindrical, radially arranged, mostly coarsely incrusted (zebroid), smooth to often scatteredly diverticulate, non-dextrinoid, up to 8.0(–12) µm wide hyphae; terminal elements and lateral projections rarely incrusted, vesiculose, conical or cylindrical, with diverticula or not, sometimes subcoralloid; incrustation dark (grey-)brown in KOH. Pileocystidia absent. Stipitipellis a cutis of cylindrical, parallel, slightly thick-walled, incrusted, smooth or scatteredly diverticulate, non-dextrinoid, up to 6.0 µm wide hyphae. Caulocystidia numerous, adpressed to erect, 18–70(–105) × (4.0–)6.0–10 µm, cylindrical, clavate, subulate, fusoid, mostly (slightly) irregular or moniliform, sometimes diverticulate, obtuse, thin-walled. Clamp connections present in all tissues.

Habitat — On dead twigs of broadleaf trees and *Pinus densiflora* in a mixed forest with dominating *Pinus densiflora, Quercus mongolica* and *Acer* sp.

Additional collections — Chuncheon, Dongsan-myeon, Bongmyeong-ri, Experimental forest of Kangwon National University, 37° 46' 46" N, 127° 48' 59" E, alt. c. 212 m, 22 July 2007 leg. V. Antonín and R. Ryoo (Antonín 07.106, 07.107, BRNM 714972 and 714973). – Ibid., 15 Aug. 2008, leg. R. Ryoo KG 247 (BRNM 721948). – Wonju, 4 July 2008 leg. J.G. Han (Antonín 08.71, BRNM 718700). – Deogyusan National Park, Cheon-yeon Falls, 24 Aug. 2007 leg. R. Ryoo KG 155 (BRNM 721947). – Heogseong, Seowon-myeon, 28 Aug. 2007 leg. R. Ryoo KG 167 (BRNM 721949). – Ibid., 21 Aug. 2008, leg. R. Ryoo KG 251 (BRNM 721950).

Remarks — *Marasmiellus koreanus* is a rather robust fungus characterised by a brownish orange, rugulose pileus (except for the centre), light yellow, broadly adnate lamellae, a long, whitish to light yellow stipe, moderately large, fusoid, ellipsoid-fusoid, or (broadly) ellipsoid basidiospores, variably shaped cheilocystidia, a pileipellis missing a Ramealis-structure, and numerous cylindrical, clavate, subulate, fusoid, mostly (slightly) irregular or moniliform, sometimes diverticulate caulocystidia. According to Singer (1973), it belongs to sect. *Dealbati* Singer, subsect. *Quercini* Singer.

Among similar species, Marasmiellus ramorum Singer is distinguished by a smaller (± 11 mm broad) pileus, a smaller (13–14 × 1 mm) stipe that is brownish below, narrower basidiospores [8.5–10.3 × 3–3.2(–4) µm)], and differently

shaped cheilocystidia (Singer 1973). *Marasmiellus enodis* Singer has a smaller (≤ 19 mm broad) brown pileus, lamellae concolorous with pileus, a shorter (7–21 × 0.5–2 mm) stipe that browns from the base, smaller basidiospores (6.5–9 × 2.5–4(–4.5) µm), and a stipe covering of *Crinipellis*-type hyphae (Singer 1973). *Marasmiellus dendroegrus* Singer is distinguished by a smaller (9–19 mm broad) striate pileus, a smaller (13–26 × 1–2 mm) stipe soon entirely cinnamon to deeply chestnut coloured, and smaller basidiospores (6–8.5 × 2.8–4.5 µm) (Singer 1973). *Marasmiellus synodicus* (Kunze) Singer has only a 3–9 mm broad pileus, a short stipe (5–8 × 0.5–1 mm), and smaller basidiospores [(3.5–)4.5–6(–6.5) × 2.2–3.5(–3.7) µm] and lacks distinct cheilocystidia (Singer 1973). *Marasmiellus stenophyllus* (Mont.) Singer is also smaller (pileus 2–15 mm broad, stipe 6–15 × 0.5–1.7 mm) and produces smaller basidiospores (6.8–8 × 2.7–3.5 µm) and different cheilocystidia (Singer 1973).

Marasmiellus rhizomorphigenus Antonín, R. Ryoo & H.D. Shin, **sp. nov.** Fig. 2

MycoBank 516551

NCBI accession numbers: BRNM 714969 [GU319115 (ITS), GU319119 (LSU)]; BRNM 715003 [GU319116 (ITS), GU319120 (LSU)]

Pileo 6–20 mm lato, late convexo, late conico usque ad planum, centro leviter depresso, ruguloso-plicato, pubescente-tomentoso, albido vel pallide griseo, centro pallide griseo-brunneo. Lamellis distantibus, albidis vel pallide luteis. Stipite 5–20 × 0.5–1.5 mm, cylindraceo vel ad basim attenuato, pubescente vel furfuraceo, apicem albido vel pallide luteo, ad basim obscure brunneo-griseo vel griseo-brunneo. Rhizomorphis praesentibus. Basidiosporis 13.5–17 × 4.5–6.5 µm, fusiformibus, clavatis vel lacrimoideis, hyalinis, inamyloideis. Cystidiis hymenialibus 34–70 × 8.0–14 µm, (sub)fusiformibus, rostratis, tenui- vel leviter crassitunicatis. Pileipellis ex hyphis cylindraceis, laevibus vel leviter incrustatis. Pileocystidiis 35–140 × 6.0–14 µm, lageniformibus, subulatis vel fusiformibus, rostratis, tenui- vel leviter crassitunicatis. Caulocystidiis 35–140 × (5.0–)6.0–12 µm, cylindraceis, subulatis, sublageniformibus, iterum rostratis, tenui- vel leviter crassitunicatis. Hyphis fibulatis, indextrinoideis. Ad ramulos putridos.

Holotypus: *Korea meridionalis, Hongcheon, Bukbang-myeon, Seongdong-ri, 27. VI. 2007 leg. V. Antonín 07.148 (**holotypus** in herbario BRNM 715003 preservatur).*

Basidiocarps single or in groups. Pileus 6–20 mm broad, broadly convex to broadly conical with obtuse or papillate centre and involute to inflexed margin when young, then ± broadly conical to almost applanate with plane to slightly depressed centre (sometimes still with obtuse papilla), and with straight to uplifted irregular margin, smooth or rugulose at the very centre, rugulose-plicate otherwise, margin crenulate, hygrophanous, translucently striate when moist, surface entirely finely pubescent-tomentose to tomentose, white or greyish tinged with pale greyish brown (6D2–3, 6E3–4) coloured centre. Lamellae distant, L = 10–18, l = 0–2, broadly adnate to shortly decurrent, lamellulae very narrow, irregular to branched, intervenose especially when old, mostly not reaching the pileus margin when old, whitish to pale yellowish

(3–4A2), sometimes with greyish tinge when old, with concolorous, finely pubescent edge. Stipe 5–20 × 0.5–1.5 mm, central, usually cylindrical and slightly broadened at apex or tapering towards base, rarely slightly broadened (up to 1.25 mm) towards base, insititious, finely fibrillose and sometimes twisted, entirely whitish pubescent to (especially at apex) furfuraceous, concolorous with lamellae at apex, brownish grey or greyish brown (6E3–4, 7E2) towards base. Rhizomorphs present, numerous, strigose, dark brown to black-brown, smooth. Context membranaceous, without special smell and taste.

Basidiospores 13.5–17 × 4.5–6.5 µm, average = 15.2 × 5.3 µm, E = 2.4–3.6, Q = 2.7–3.3, fusoid, lacrimoid, clavate, sometimes curved, smooth, hyaline, thin-walled, non-dextrinoid. Basidia 43–52 × 11–15 µm, 1-, 2-, 3- and 4-spored (4-spored ones seem to be the most frequent), clavate. Basidioles 25–52 × 5.0–10(–16) µm, cylindrical or (broadly) clavate. Hymenial cystidia 34–70 × 8.0–14 µm, fusoid, (sub)lageniform, rostrate, obtuse, thin- to slightly thick-walled, hyaline. Trama hyphae cylindrical to subinflated, thin- to slightly thick-walled, hyaline, non-dextrinoid, up to 20 µm wide. Pileipellis a cutis composed of cylindrical, radially arranged, thin- to slightly thick-walled, smooth or minutely incrusted, non-dextrinoid, up to 12 µm wide hyphae; terminal cells ± cylindrical, regular or irregular, thin-walled. Pileocystidia 35–140 × 6.0–14 µm, lageniform, subulate, fusoid, rostrate, obtuse, thin- to slightly thick-walled (walls up to 0.75 µm). Pileosetae absent. Stipitipellis a cutis of cylindrical, parallel, thin- to slightly thick-walled, smooth or minutely incrusted, non-dextrinoid, up to 7.0 µm wide hyphae. Caulocystidia adpressed to erect, 35–140 × (5.0–)6.0–12 µm, cylindrical, subulate, sublageniform, mostly slightly irregular or submoniliform, often rostrate, obtuse, thin- to mostly slightly thick-walled (walls up to 0.5 µm). Rhizomorph hyphae cylindrical, thick-walled, smooth, up to 4.0 µm wide, yellow-brown in KOH in cortex, similar but hyaline in medulla. Clamp-connections present in all tissues.

Habitat — On dead twigs of *Larix* sp., *Castanea serrata, Quercus mongolica, Alnus* sp. and a broadleaved tree (*Quercus*?) in mixed forests.

Additional collections — Chuncheon, Dongsan-myeon, Bongmyeong-ri, Experimental forest of Kangwon National University, 37° 46' 46" N, 127° 48' 59" E, alt. c. 212 m, 22 July 2007 leg. V. Antonín and R. Ryoo (Antonín 07.99, BRNM 714969). – Ibid., 15 July 2009 leg. V. Antonín and R. Ryoo (Antonín 09.100, BRNM 718759). – Hongcheon, Gongjaksan Ecological Park, 16 July 2009 leg. V. Antonín and R. Ryoo (Antonín 09.111, BRNM 718771). – Guri, Donggureung (Nine East Tombs), 37° 36' 59" N, 127° 07' 56" E, alt. c. 35 m, 11 July 2009 leg. V. Antonín and R. Ryoo (Antonín 09.70, BRNM 718731).

Remarks — *Marasmiellus rhizomorphigenus* is characterised by having a greyish to whitish pileus, irregular to branched lamellae that are intervenose especially when old, a short stipe often tapering towards base that is concolorous with lamellae at the apex and brownish grey or greyish brown towards base,

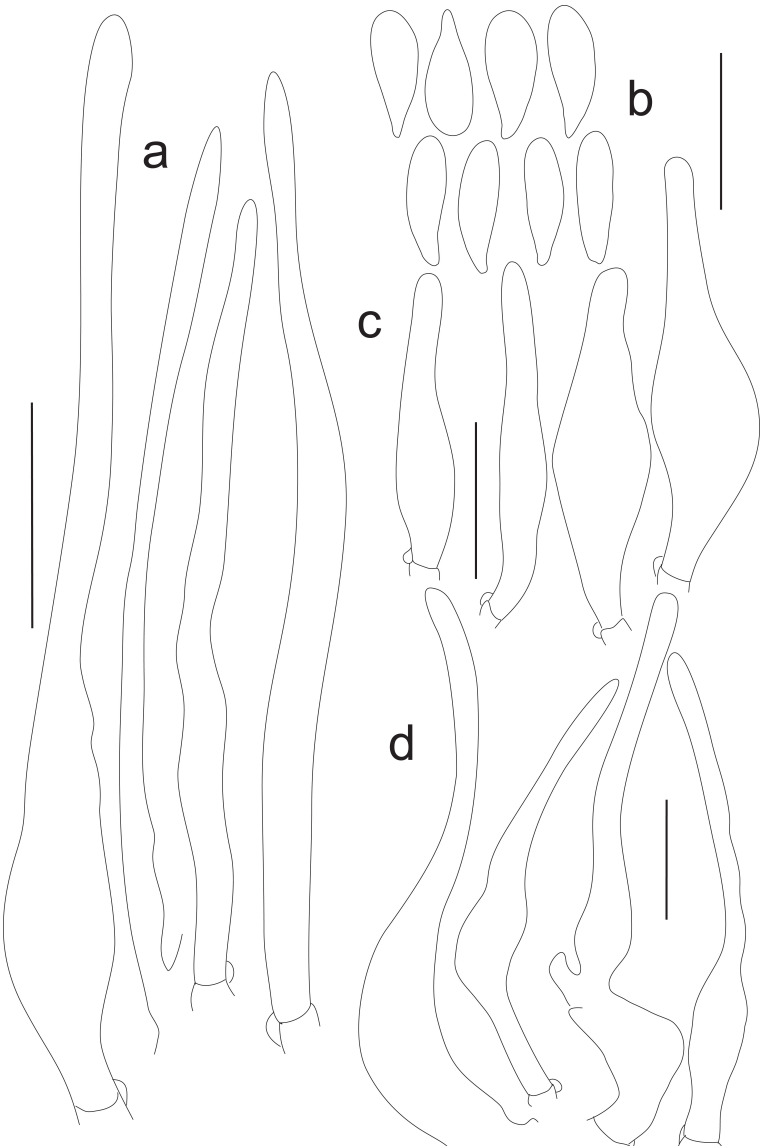

Fig. 2. *Marasmiellus rhizomorphigenus.*
a. caulocystidia, b. basidiospores, c. hymenial cystidia, d. pileocystidia.
Scale bar = 20 μm.

well-developed rhizomorphs, rather large basidiospores, well-developed fusoid or (sub)lageniform hymenial cystidia, the presence of pileocystidia, and the absence of pileosetae. These characters place it in *Marasmiellus* sect. *Candidi* Singer according to traditional systematics (Singer 1973).

The macroscopically very similar species *M. candidus* (Bolton) Singer especially differs by the absence of rhizomorphs and distinct pileocystidia (Antonín & Noordeloos 2010); this species has already been recorded from the Korean Peninsula (Wojewoda et al. 2004). On the other hand, the fungus published as *M. candidus* with a photo by Park & Lee (1991) represents our *M. rhizomorphigenus*. *Tetrapyrgos nigripes* (Schwein.) E. Horak differs by the shape of its basidiocarps (stipe longer than pileus diameter), the tetrahedric shape of the basidiospores, a different pileipellis structure, and the absence of rhizomorphs and setoid pileocystidia. *Marasmiellus albofuscus* (Berk. & M.A. Curtis) Singer has a reticulate-sulcate pileus, a pallid to white stipe, and slightly smaller basidiospores (10.8–15.3 × 3.5–6.2 µm). *Marasmiellus subnigricans* (Murrill) Singer has a larger (15–40 mm), white pileus that ages or dries to deep fuscous or blackish, often blackish lamellae, a white stipe becoming black-punctate, and smaller basidiospores (10.2–14.5 × 3.5–4.3 µm). Moreover, neither *M. albofuscus* nor *M. subnigricans* form rhizomorphs (Singer 1973).

No previously described rhizomorph-forming species (Desjardin et al. 1993, Singer 1973) belongs to sect. *Candidi*. The macroscopically very close *Marasmiellus tenerrimus* (Berk. & M.A. Curtis) Singer differs by a pileus that appears finely cinnamon punctate under a lens, a smaller stipe (5–12 × 0.3–0.4 mm), shorter hymenial cystidia (20–27 × 4–6 µm), and the presence of pileosetae (Desjardin et al. 1993, Singer 1973).

Phylogenetic analyses

The phylogenetic relationships of *Marasmiellus koreanus* and *M. rhizomorphigenus* were inferred from Bayesian (MCMC) analyses based on internal transcribed spacer (ITS) and nuclear ribosomal large subunit (LSU) rDNA sequences obtained in this study and from GenBank. ITS and LSU sequences were aligned and the ends trimmed to create a dataset of 561 and 797 base pairs, respectively. The resulting phylogenetic trees are shown in Fig. 3 (ITS) and Fig. 4 (LSU).

The phylogeny inferred from LSU and ITS sequences support the isolated position of the species delimited by macro- and micro-morphological characteristics. The independent taxonomic status of the two new *Marasmiellus* species in relation to other closely related species was concordant with high posterior probability. The results of this study were supported by the phylogenetic relationships and placement of *Marasmiellus* s.l. in previous studies by Mata et al. (2004) and Wilson & Desjardin (2005).

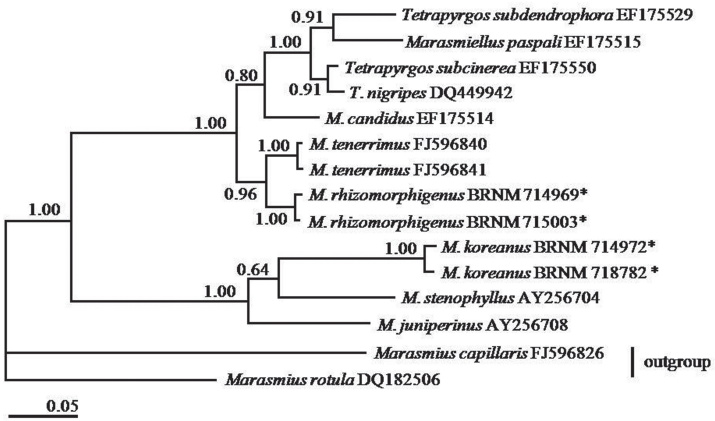

Fig. 3. Phylogenetic tree of *Marasmiellus koreanus* and *M. rhizomorphigenus* based on ITS rDNA sequences, showing mean branch lengths of a 50 % majority-rule consensus tree from a MCMC analysis. An asterisk (*) denotes taxa sequence on this study. The bar indicates number of expected substitutions per position.

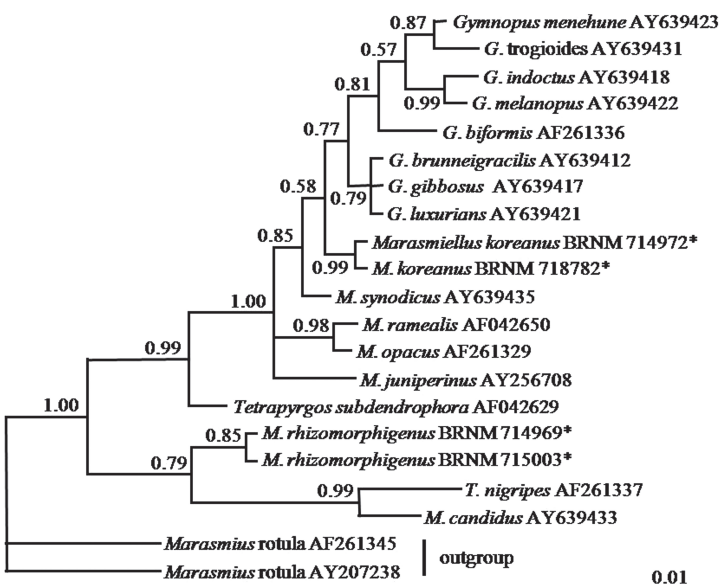

Fig. 4. Phylogenetic tree of *Marasmiellus koreanus* and *M. rhizomorphigenus* based on the nLSU rDNA sequences, showing mean branch lengths of a 50 % majority-rule consensus tree from a MCMC analysis. An asterisk (*) denotes taxa sequence on this study. The bar indicates number of expected substitutions per position.

Both ITS and LSU sequences place *Marasmiellus koreanus* in the same clade with *M. juniperinus* (the type species of *Marasmiellus*) and *M. stenophyllus* (ITS) and *M. synodicus*, *M. ramealis,* and *M. opacus* (LSU). According to the phylogenetic analyses of Mata et al. (2004) and Wilson & Desjardin (2005), *Marasmiellus juniperinus* belongs to the same clade as species of *Gymnopus* sect. *Vestipedes*. The proposed transfer of *M. juniperinus* to the genus *Gymnopus* by Mata et al. (2004) was not accepted by Wilson & Desjardin (2005). This study confirms the placement of *Marasmiellus koreanus* in the /marasmiellus clade according to Wilson & Desjardin (2005).

The other new species, *Marasmiellus rhizomorphigenus*, forms a distinct sister branch to *Tetrapyrgos* taxa in two phylogenetic trees. This species is placed in the same clade with *Marasmiellus tenerrimus* from ITS analysis and with *M. candidus* from LSU analysis. According to Moncalvo et al. (2002), the /tetrapyrgos clade from the upper /tetrapyrgoid clade forms a sister clade of /marasmioid; both upper clades belong to /marasmiaceae. Analogous results were published by Matheny et al. (2006). Nevertheless, Wilson & Desjardin (2005) excluded the /tetrapyrgos clade from /marasmiaceae, which corresponds to the /marasmioid clade of Moncalvo et al. (2002). In general, the phylogenetic positions of *Marasmiellus, Tetrapyrgos,* and *Gymnopus* sect. *Vestipedes* deserve further study.

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